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13 ABSTRACT

Red cell electrophoretic mobilities have been determined on cells from 6 baboons subjected to hemorrhagic shock and 2 baboons in E. coli shock. Studies of cells suspended both in saline and in their own plasma showed electrophoretic mobility in hemorrhagic shock to be unchanged from control values. Power function analysis showed the maximum undetectable change for cells measured in saline would be 6.7%, and in plasma 12.2%. Use of either heparin or EDTA as anticoagulant, or absence of anticoagulant, did not affect the mobilities of either control or shock cells. It is concluded that loss of red cell electronegativity is not an important factor in producing red cell aggregation in hemorrhagic shock, but that this may be of significance in the septic shock.

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Studies on the Effect of Shock on Red Cell Surface Charge in Primates[1 2]

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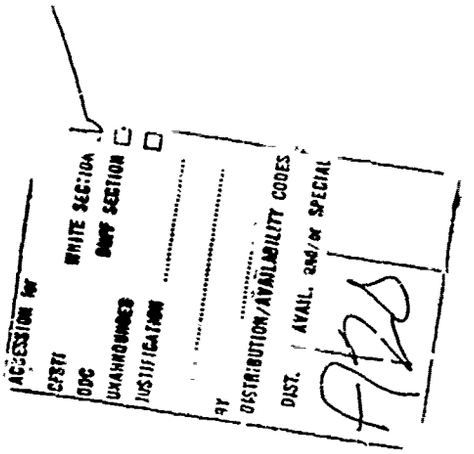

Introduction

The phenomenon of erythrocyte aggregation or sludging has been observed in a number of types of shock, including septic shock and traumatic shock, as reviewed by KNISELY [1965]. Although KNISELY [1965] has stated that 'simple hemorrhage' does not give rise to intravascular red cell aggregation, recent microscopic observations by VANECKO *et al.* [1969] have revealed progressive erythrocyte aggregation in Rhesus monkeys subjected to hemorrhagic shock with arterial pressures of 20–60 mm Hg.

In vivo red cells are known to repel one another [see KNISELY *et al.*, 1960] and numerous investigators have asked why this property is lost in shock. BELLIS and SNOW [1950] have suggested that agglutination of erythrocytes is due to lowered electrical surface charge on individual cells, thus enabling adsorption of cells onto each other. It is the purpose of this report to look at the possible loss of electrostatic repulsion as a factor in producing the red cell aggregation observed in shock.

Methods

Healthy male baboons (*Papio doguera*) were used after a 30-day quarantine period. On the day of study, they were sedated with phencyclidine hydrochloride (Sernylan) in a dose of 1 mg/kg, and catheters were placed in the femoral artery and vein. Samples of blood were collected in plain glass test tubes, and in tubes containing sodium heparin (143 USP units/10 ml blood) or disodium EDTA (12 mg/10 ml blood). The blood was promptly diluted 1:100 in isotonic phosphate-buffered saline at pH 7.4 and electrophoretic mobility determined within 15 min of the time of blood collection. Another set of blood samples was centrifuged to separate cells from plasma (or serum) and the cells were resuspended in their own plasma (serum), again in a 1:100 dilution. The animals were then bled to a mean arterial pressure of 60 mm Hg and maintained at that pressure for 1 h; Ringer's lactate solution was given as needed to maintain the pressure at that level. After 1 h, the pressure was lowered to 40 mm by further bleeding and maintained at this level for an additional hour. Blood samples were obtained as before.

Two animals were also studied in septic shock, induced by i.v. injection of live *E. coli* in a dose of 10^{11} organisms per kilogram. The *E. coli* were grown from a strain of known serotype under constant conditions, and this dose was found to uniformly bring the animal's mean arterial pressure below 60 mm Hg within 1 h. Samples were collected before and 1 h after injection of *E. coli*.

Electrophoretic mobility was determined in a

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2 The experiments were conducted according to the principles enunciated in "Guide for Laboratory Animal Facilities and Care".

3 The opinions or assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

commercially available Northrup-Kunitz cataphoresis apparatus (A. H. Thomas Co.) similar to that described by ABRAMSON *et al.* [1942]. The time for red cells to migrate 0.04 mm in an electrical potential gradient of 2.86 V/cm was determined for 10 cells from each sample. All measurements were made in a room maintained at 25 °C ± 1. Plasma and serum viscosities were measured in a cone-plate microviscometer (Brookfield Laboratories, Inc.) at shear rates of 10–100 sec^{-1}, on serum and on plasma collected in heparin and EDTA at concentrations similar to those described above.

Erythrocyte surface charge is related to electrophoretic mobility by the equation $Q = 6\pi r\eta(v/E_x) \cdot 10^{-7}$, where Q is the charge in C, 6π is a proportionality factor from Stokes' Law, r is the radius of the red cell, η is the viscosity of plasma, and (v/E_x) is the electrophoretic mobility (velocity/potential gradient). 10^{-7} is a factor to convert the final result to C. The formula must be considered an approximation as the factor 6π is exact only for a sphere.

Results

Table I shows the electrophoretic mobilities of red cells obtained from 6 separate animals before and after 2 h of hemorrhagic shock as determined from red cells collected without anticoagulant and with either heparin or EDTA. It can be seen that in no case does the shock cause a statistically significant difference (at the $P = 0.05$ level). A 3-way analysis of variance, comparing the effects of anticoagulant, shock, and animal variation, shows that all of the observed variations in red cell mobilities can be accounted for by variation within replicates and by variation between animals. Neither shock nor anticoagulant (or absence of anticoagulant) accounts for any of the observed differences. For this reason, data for the non-anticoagulated, heparinized, and EDTA-treated blood have been pooled into the 'overall' averages given in Table I. To detect any changes in erythrocyte charge which might be related to loosely adsorbed plasma components, mobilities of cells in their own serum or plasma were determined. These are also given in Table I. Again, no significant differences are observed, although EDTA measurements border on significance at the $P = 0.05$ level. A 3-way analysis of variance again indicated that neither anticoagulant nor shock contributed to net variation.

Table II compares data from the 2 animals subjected to *E. coli* septicemia with the hemorrhagic shock animals. We find that while red cells in buffered saline show the same mobilities in both types of shock, there is a substantial decrease in mobility in the *E. coli* shock cells when measured in plasma, as compared to hemorrhagic shock. Although this difference is statistically quite significant, with $P < 0.005$, it must be interpreted cautiously because of the very small number of animals studied, especially in view of substantial animal-to-animal variability.

Measurements of plasma and serum viscosity showed considerable animal-to-animal variation, and the values can hence not be determined with great precision. The viscosities, which were essentially independent of shear rate, were 1.3 ± 1.08 cP (±S.E. of mean) for control serum, and 1.4 ± 1.08 cP for serum from hemorrhagic shock animals, a statistically

Table I. Mobility of red cells in saline and plasma in hemorrhagic shock

Anti-coagulant	State	Mobility ± S.E., μm/sec/V/cm	
		In saline	In plasma
None	Control	1.33 ± 0.043	1.27 ± 0.094
	Shock	1.35 ± 0.063	1.33 ± 0.060
Heparin	Control	1.29 ± 0.036	1.24 ± 0.071
	Shock	1.24 ± 0.039	1.24 ± 0.060
EDTA	Control	1.35 ± 0.033	1.51 ± 0.087
	Shock	1.38 ± 0.040	1.24 ± 0.072
Overall	Control	1.32 ± 0.019	1.34 ± 0.070
	Shock	1.30 ± 0.035	1.35 ± 0.049

Table II. Red cell mobility in hemorrhagic and septic shock, as determined in saline and plasma

Type of shock	Mobility ± S.E., μm/sec/V/cm	
	In saline	In plasma
Hemorrhagic	1.30±0.035	1.35±0.049
Septic	1.37±0.049	1.13±0.049
P*	n. s.	0.005

* P values based on Student 't' test

non-significant difference. Similarly, the viscosity was not significantly different in samples collected with heparin, EDTA, or without anticoagulant.

Plasma pH was determined before resuspension of red cells. Although all shock animals became acidotic as determined by anaerobic blood pH measurement, the plasma became somewhat alkalotic when exposed to room air. The pH in room air was found to be 7.7±0.09 for control samples, and 7.5±0.08 for shock animals.

Discussion

Our measurement of red cell mobility in saline, 1.32±0.019 μm/sec/V/cm, is in good agreement with the values given by ABRAMSON *et al.* [1942] for man (1.31 μm/sec) and Rhesus monkey (1.33 μm/sec). Our calculated values for red cell charge are $(1.74\pm0.023)\times10^{-15}$ C for red cells in saline, and $(1.76\pm0.064)\times10^{-15}$ C for red cells in their own plasma. This latter value is in good agreement with that of BERNSTEIN and CASTANEDA [1965] who found that human red cells in plasma have a net charge of 1.6×10^{-15} C.

In evaluating the outcome of an experiment whose results may be considered 'negative' in the sense that the sought for effect was not observed, we must ask whether our methodology was sensitive enough to detect this effect. Statistically, this is done by means of a 'power function', which gives the probability of rejecting the null hypothesis (that is, the hypothesis that red cell mobility is unchanged in shock) when the actual change in red cell mobility is a given value. Such an analysis shows that a change in mobility of 0.088 μm/sec/V/cm, or 6.7% of control, will be detected with 95% certainty. From this, we may conclude that red cell mobility in hemorrhagic shock does not change by more than 6.7%. A similar analysis for the erythrocytes in plasma indicates that a change in mobility of 0.163 μm/sec., or 12.2% would be detected with 95% confidence.

We may also ask whether erythrocyte mobility, the quantity actually determined, is directly related to the cell net charge, the desired quantity. We note that, in addition to mobility, the viscosity of the suspending medium enters into the calculation of surface charge. Viscosity does not change at all in the case of red cells in buffered saline, which was of the same composition throughout the experiment. Also, our studies indicated no significant change in plasma viscosity during shock. The well-known changes in whole blood viscosity in shock do not affect our studies, in which the red cell suspension is sufficiently dilute that only plasma viscosity need be considered. Thus, we may consider that electrophoretic mobility is a direct measure of cell charge, and we may set maximum limits of 6.7 and 12.2% for the change of erythrocyte surface charge in hemorrhagic shock as measured in saline and plasma, respectively.

We therefore conclude that erythrocyte aggregation observed in hemorrhagic shock is unlikely to be due to changes in cell surface charge. OSGLEY [1965] reached a similar conclusion using a rather different approach based on iso-hemagglutin in-

duced aggregation. However, HISSEN *et al.* [1966] have reported that heparinized dogs subjected to hemorrhage show a net decrease of red cell surface charge averaging 25% for erythrocytes suspended in plasma, but not for those in saline. This difference from our results could possibly be attributed either to species difference or to the prior *in vivo* heparinization. Finally, the possibility of decreased surface charge promoting red cell aggregation in septic shock remains, and is especially interesting in view of electron microscopic observations by MERGENHAGEN *et al.* [1969] of physical defects in the red cell surface after treatment with endotoxin.

Summary

Red cell electrophoretic mobilities have been determined on cells from 6 baboons subjected to hemorrhagic shock and 2 baboons in *E. coli* shock. Studies of cells suspended both in saline and in their own plasma showed electrophoretic mobility in hemorrhagic shock to be unchanged from control values. Power function analysis showed the maximum undetectable change for cells measured in saline would be 6.7% and in plasma 12.2%. Use of either heparin or EDTA as anticoagulant, or absence of anticoagulant, did not affect the mobilities of either control or shock cells. It is concluded that loss of red cell electronegativity is not an important factor in producing red cell aggregation in hemorrhagic shock, but that this may be of significance in the septic shock animal.

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